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A Study of Experimentally Induced Endocytosis in a Teleost. I. Light Microscopy of Peripheral Blood Cell Responses.

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(Plates I-III; Text-figure 1)

The peripheral blood picture of the goldfish, *Carassius auratus*, was studied to determine cell functions and analogies to mammalian cell types. Blood samples were collected at intervals, over a 26 hour period, from fish which had received a single intraperitoneal injection of Thorotrast. Stained blood smears were examined for qualitative and quantitative changes. The mean ± S. E. and 95% range were determined for all leukocytes. Responsive elements were lymphocytes, immature and mature neutrophils, and a variety of blast cells. Lymphopenia and neutrophilia persisted for six hours. All neutrophilic maturation stages were present. The reaction decreased after 12 hours, and the blood picture began to normalize after 18 hours. The rare macrophages did not respond. Mononuclear phagocytes were limited to lymphocytes and blast cells. The lymphoid hemoblasts are analogous to mammalian macrophages studied under similar conditions. Immature blood cells of the teleost have the potential for endocytosis as well as cell proliferation and differentiation. Consideration is given to the probable role of the lymphocyte in hemopoiesis and endocytosis.

Introduction

The peripheral blood cells and hemopoietic tissues of the goldfish, *Carassius auratus*, have been studied to further elucidate problems which still exist in comparative hematology. These problems concern the origins of the various cell types, their interrelationships, and their functions under both normal and pathological conditions. The phylogeny of leukocytes, and the analogies existing between cells of lower vertebrates and mammals, are of particular interest.

Prior hematological studies of teleosts have included goldfish (Loewenthal, 1927; Watson, 1963; Weinreb, 1963), perch (Yokoyama, 1960), carp and brook trout (Dombrowski, 1953), salmon (Katz, 1950; Watson *et al.*, 1956), and rainbow trout (Weinreb, 1958). Earlier accounts of hematological reactions in poikilothermic vertebrates have involved such experimental conditions as injected foreign matter (Mesnil, 1895; Metchnikoff, 1905; Wislocki, 1917; Mackmull and Michels, 1932; Easton, 1952), acute inflammation (Weinreb, 1958; Yokoyama, 1960), viral infection (Watson, *et al.*, 1956), bacterial infection (Katz, 1950), and x-irradiation (Watson, 1961; Schechmeister, *et al.*, 1962). In all these studies consideration was given to the role of peripheral blood cells as indicators of systemic responses associated with phagocytosis and acute inflammation. Such responses, particularly notable in circulating blood cells, are probably universal among animal cells. The specific reactive cell types in the lower vertebrates, however, remain to be more clearly defined and the mechanics of cellular responses require further elaboration.

The purpose of this study was to determine the specific responses of a teleost to experimentally induced endocytosis, as manifested by the circulating blood cells, to define more clearly the problems of blood cell function and comparative hemopoiesis. Blood cells were evaluated by correlated light and electron microscopic studies based on previously established morphological criteria (Jakowska, 1956; Watson, 1963; Weinreb, 1963). Appraisal of the total peripheral blood picture, including distinction between the various immature and mature leukocytes,

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based on staining reactions and quantitative analysis, were necessarily accomplished by light microscopy. The mechanics of endocytosis and the fine structure of responsive cell types, as revealed by electron microscopy, will be reported separately.

A preliminary report of the study was presented at the 64th Annual Meeting of the American Association for the Advancement of Science (Weinreb and Weinreb, 1967).

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Materials and Methods

Goldfish of both sexes, ranging in size from 7 to 20 cm in length, were maintained in well-aerated tanks with a water temperature range of 22°-25°C. The same water temperature range has been used for all the goldfish hematological studies, since total erythrocyte and leukocyte counts are known to vary according to the ambient temperature (Slicher, 1927; Spoor, 1951). Both control and experimental animals were maintained for a minimum of two weeks before use in order to acclimatize to laboratory conditions. Experimental fish received a single intraperitoneal injection of Thorotrast (Testagar Co., Inc.) in doses of 0.1 ml to 0.3 ml, according to size, and subsequently sacrificed after intervals of 1, 3, 5, 6, 12, 18-19, and 24-26 hours following injection.

Peripheral blood samples were collected directly from the haemal vessels after severing the caudal peduncle. No anticoagulants or other reagents were used. Air-dried blood smears were stained with Wright's stain for study by bright-field microscopy. Wet mounts of live blood cells were prepared for phase-contrast study (Weinreb, 1958). Stained blood smears were studied for both qualitative and quantitative changes. The blood picture was examined for responsive cell types and morphological evidence of endocytosis. Differential leukocyte counts of mature and immature stages, in both control and experimental fish, were made. Since the variability in teleost counts is great, it was necessary to establish a "normal" standard. The mean ± standard error (S. E.) and the range of mean, where range is the distance between the upper and lower 95% confidence limits, were determined for every cell type in the control group. The mean ± S. E. and range of mean for the corresponding cell type in each experimental group were determined and compared to the "normal" standard values. Data were considered statistically significant where two ranges failed to overlap.

Animals were routinely autopsied for evidence of gross lesions, particularly at the hemopoietic sites. Wet mounts, and Wright's-stained dried smears, of the original Thorotrast preparation were examined by phase-contrast and bright-field microscopy.

Observations

Examination of fish autopsied immediately following collection of blood samples indicated no observable gross lesions, or injection site inflammation. Phase-contrast observations of the wet mounts revealed colorless to yellow, refractile, particulate matter in the plasma and within the cells. In the Wright's-stained preparations, similar material appeared as red, refractile particles or granules. This material was identified as the endocytic agent, Thorotrast. Phase-contrast studies of live cells, however, were less satisfactory than the bright-field studies of stained smears and served mainly to confirm findings.

The goldfish possesses a highly differentiated blood picture. The mature leukocytes always present in the circulation include lymphocytes, neutrophils, thrombocytes, and small numbers of eosinophils and basophils. All stages in leukocytic and erythrocytic maturation from stem cell to mature cell types, excluding consideration of the thrombocyte, are also normally present. However, the incidence of different intermediate cell types noted in the circulation is normally low, since the early developmental stages are found mainly in hemopoietic tissues, such as head kidney. The hemocytoblast is the most immature cell observed, followed by the large and small lymphoid hemoblasts, which are precursors of the leukocytic and erythrocytic series. The more mature lymphoblast is closely identified with the lymphoid hemoblasts.

Since the morphological criteria for identification of the different levels of blast cells overlap to a great extent, and represent a maturation continuum, it is more advantageous to consider these levels (hemocytoblast, lymphoid hemoblasts, lymphoblast) grouped together as "blast" cells. Most of the blast cells noted in stained blood smears are lymphoblasts, or intermediate stages between lymphoid hemoblast and lymphoblast; earlier stages are relatively rare in the circulation. The rare macrophages are also normally present. Monocytes are not found in the goldfish.

In normal peripheral blood the mean ± S. E. and range of mean (95% range) for leukocytes are as follows: lymphocyte, 77.08 ± 2.94, (71.12-82.88); neutrophil, 8.64 ± 1.83, (4.98-12.30); eosinophil, 1.92 ± 0.70, (0.78-2.06); basophil, 1.07 ± 0.45, (0.17-1.97); thrombo-

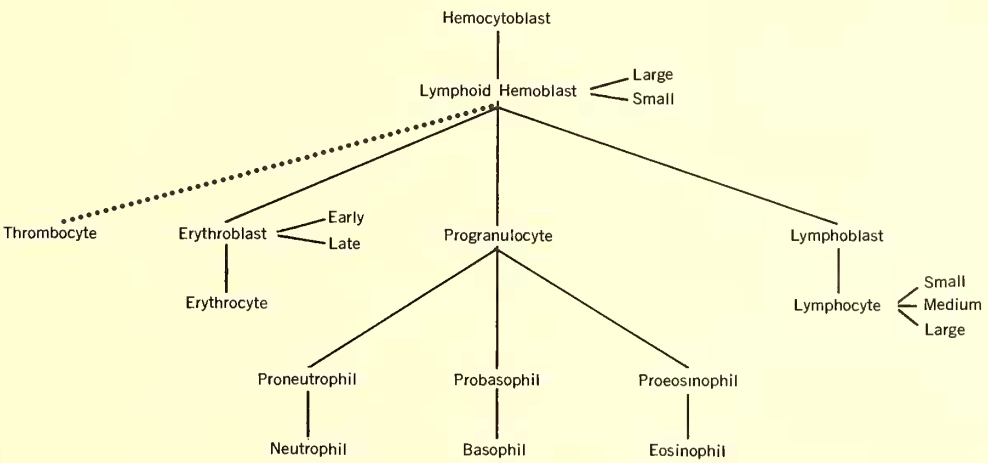
cyte, 8.00 ± 2.50, (3.00-13.00); blast cells, 2.50 ± 0.41, (1.68-3.32); macrophage, 0.50 ± 0.44, (0.38-1.38); progranulocyte, 0.29 ± 0.20, (0-0.69).

An hemopoietic scheme for the goldfish, indicating the cell types observed and the maturation sequence, is outlined in Text-figure 1. The lymphocyte is the predominant leukocyte under control conditions. The various maturation stages of the neutrophil are not prominent in the circulation and are generally referred to as proneutrophils or immature neutrophils. In the experimental animals studied, however, the entire sequence in neutrophilic maturation was evident, and the cell types may be compared to various stages in mammalian myeloid development.

Examination of the blood samples collected one hour after injection revealed an abundance of both mature and immature neutrophils. The immature neutrophils resemble mammalian myelocytes and metamyelocytes (Pl. I, figs. 1, 2). The less mature neutrophils, in contrast to the mature cells, are larger; there are varying degrees of basophilia throughout the slightly acidophilic cytoplasm, and the nucleus is larger. In addition, two types of granulation were visible; large, acidophilic, refractile granules, and finer, more flocculent, neutral granules. The striking feature in many of these cells was the presence of large, round, red, refractile inclusions in the cytoplasm. These inclusions, distinct from the cytoplasmic granulation, by virtue of their size and coloration, were phagocytosed aggregates of the marker, Thorotrast. Such inclusions were particularly prominent in the perinuclear or "hof" area. The immature neutrophils, therefore, exhibited three types of cytoplasmic structures: larger, light red, refractile granules; smaller, finer, neutral granules; phagocytosed foreign material. Lymphocytes, of medium to large variety, were present instead of the normally predominant small, round cells with pycnotic nuclei. These agranulocytes may exhibit extensive pseudopod formation. Occasional eosinophils, demonstrating evidence of phagocytosis and uptake of Thorotrast, were also noted.

The same blood picture persisted at three hours, with the appearance of prominent vacuolization of the neutrophilic cytoplasm. Cytoplasmic vacuolization, devoid of debris, was also conspicuous in the lymphoid hemoblast (Pl. I, figs. 3, 4). The large, lymphoid hemoblast in figure 4, showing areas of decreased basophilia, closely resembles the mammalian monocyte in morphology and cytoplasmic staining. Large basophilic cells, with immature nuclei and prominent nucleoli, represent the lymphoblast stage (Pl. I, fig. 5) also conspicuous at this time. Lymphocytes, which appeared to have undergone division, were noted (Pl. I, fig. 6; Pl. II, fig. 7); the binucleate condition of the lymphocyte, observed in figure 7, is not an uncommon feature in goldfish. One blood sample showed an over-abundance of developing erythrocytes, particularly basophilic erythroblasts, and a paucity of leukocytes (Pl. I, fig. 5).

Five and six hour samples exhibited the continued response of neutrophils, lymphocytes, and blast cells. Neutrophilia and lymphopenia, noted during the first hour, persisted, and the number of blast cells, mainly small hemoblasts and lym-



TEXT-FIG. 1. Hemopoietic scheme of goldfish, indicating cell types present, and their maturation sequence.

phoblasts in varying stages of development, were markedly increased (Pl. II, figs. 8, 11). Blast cells, like neutrophils and lymphocytes, are vacuolated; the granular inclusions observed are indicative of phagocytosed foreign material rather than cytoplasmic granules. Among the more mature lymphocytes, some appear to be relatively inactive, while others, characterized by numerous pseudopodia and cytoplasmic inclusions, appeared to have been stimulated. Large vacuoles within lymphocyte cytoplasmic blebs, filled with amorphous, acidophilic material, resemble acidophilic lakes (Pl. II, fig. 12; Pl. III, fig. 13). Neutrophilic stages at this time are comparable in maturation to mammalian myelocytes and metamyelocytes in which the cytoplasmic granulation is readily identifiable (Pl. II, figs. 8, 9, 10). Inclusions, vacuoles, and granules are prominent in the goldfish "metamyelocytic" stage (Pl. II, fig. 10). A rare macrophage was found in a five hour sample. Although large cytoplasmic vacuoles were prominent, no evidence of engulfed foreign matter was noted.

At 12 hours, the total peripheral blood picture appeared to be normalizing. Previously abundant neutrophils and blast cells were less prominent. The neutrophils present are comparable to mammalian metamyelocytes and mature cell types. Phagocytic activity was still noted. Lymphocytes, although still below the normal range, were on the increase. The majority demonstrated mature morphology and were medium to small in size. Blast cells were represented by late lymphoblasts. There was also evidence of a rise in thrombocytes.

After 18-19 hours, the only blast cells noted were late stages in lymphoblast development, which, with the increase of mature lymphocytes, is indicative of maturation in the lymphocytic series. Little evidence of phagocytosis was seen, with the exception of slight vacuolization in some blast cells. Proneutrophils (comparable to mammalian myelocytes) and progranulocytes were also noted. An unusually high incidence of basophilic granulocytes, with a scarcity of other granulocytes, was observed in one blood sample. Probasophils, immature basophils, and mature basophils were present (Pl. III, figs. 14, 15, 16).

Twenty-four to 26 hours after injection, the blood picture had essentially returned to normal. Cell types noted were: lymphocytes, of various shapes and sizes; neutrophils; and the usual incidence of immature cells. The variety in lymphocyte morphology resembled the typical picture noted in control animals. Small numbers of late lymphoblast stages (Pl. III, fig. 17), as well as medium and large lymphocytes, were present in addition to the prevalent small variety (Pl. III, fig. 18). Neutrophils demonstrated rare vacuolization and signs of phagocytosis. Throughout the 26 hours during which the reactions were followed, the normally low incidence of macrophages did not change significantly.

Since the responsive cell types in all experimental groups were the lymphocytes, neutrophils, and blast cells, the mean± S. E. and range of mean are given only for these cell types. Table I compares the values between cells in control and experimental groups, at each time interval. Significant changes occurred in lymphocyte and neutrophil counts after one, five, and six hours, and in blast cells after three and six hours.

Discussion

Light and electron microscopy, because of wide differences in magnification and resolution levels, reveal different degrees of cellular response to foreign agents. The resolution limits of the light microscope restrict observation on the engulfment and incorporation of material into the cell to phagocytosis and pinocytosis. At the ultrastructural level, however, the details of micropinocytosis are revealed. These responses have in common a basic process whereby engulfed material is first surrounded by cell membrane prior to incorporation into the cell and is best described by the term "endocytosis" (de Duve, 1963). Thorotrast has proven particularly useful in studies of endocytosis by virtue of its properties of serving as both stimulus and marker. Although resolution of single Thorotrast particles requires electron microscopy, aggregates can be observed by light microscopy.

The predominant problems noted in reports on teleost hematology center on the agranulocytic, or mononuclear, cells. The reference to mononuclear cells, or mononuclear phagocytes, in both comparative and mammalian studies, encompasses many cell types. In the mammalian frame of reference, the cells include the various lymphocytes, monocytes, macrophages, and tissue cells (*e.g.*, epithelioid and giant cells) which comprise the cells of the reticulo-endothelial system. No such clear-cut identifications exist in the teleost. The cell types enumerated are not sharply defined and a reticulo-endothelial system is not as clearly delineated morphologically or physiologically. The overlap in cell morphologies, and relatively random distribution of blood and tissue phagocytes in the teleosts, lead to less readily identifiable and localized responses. Definition of teleost mononuclear cells, and analogies to well-studied mammalian cell types, should be considered.

Differences in average cell counts of the agranulocytes, particularly the predominating

lymphocytes, in goldfish result from confusion in identification of the various lymphocytes, blast cells, and questionable monocytes. Loewenthal (1928, 1930) reported 72.6% lymphocytes and 8.6% monocytes, whereas, Watson (1963) reported a total lymphocyte count of 92.5%. In the former report, hemoblasts and large lymphocytes seem to have been classified as monocytes. From the present study, as well as that of Watson, it is concluded that a cell corresponding to the mammalian monocyte is lacking in the goldfish. Jakowska (1956) claimed large lymphoid hemoblasts of the teleosts resembled human monocytes, and indicated that several cell types considered to be monocytes were probably large and small hemoblasts. The mononuclears of the goldfish, therefore, should refer only to lymphocytes, macrophages, and the various blast cells.

The peripheral blood picture is further complicated by the presence of overlapping stages in mononuclear cell development. Transformations from one cell type to another are also known to occur in normal (healthy) fish. Such changes are observed to a greater degree in pathological and certain experimental conditions. Therefore, the relative values for mononuclear cells are variable and indicative of the dynamic state of the peripheral blood picture.

Lewis and Lewis (1926) observed, in hanging-drop blood cultures from lower vertebrates over a period of several days, that mononuclear cells appeared to increase, although no cell divisions were noted. Forms in transition to clasmatocytes and epithelioid cells were seen. They concluded that large mononuclears, macrophages, and epithelioid cells were merely different phases of the same cell type; that observed so-called "transformations" of mononuclears into macrophages, clasmatocytes, epithelioid cells, and Langhans-type giant cells were not to be considered as true differentiation into new cell types, but rather in the nature of temporary, functional variations of the same cell type; that morphological differences noted depended upon the degree of phagocytic activity, during which the amount and character of ingested material, the degree of digestion, and assimilation of foreign matter varied. Although the mononuclear cell was considered morphologically distinct from lymphocytes and granulocytes, Lewis and Lewis noted that, in most of the species studied, there are blood cells which appear to bridge the gap between the mononuclears and lymphocytes.

Loewenthal (1927, 1928) described the agranulocytes of goldfish as lymphocytes and large mononuclear cells, comparing the latter to transitional forms in higher animals, which he indicated, became granular leukocytes. Yoko-

TABLE I

DIFFERENTIAL LEUKOCYTE COUNTS FOLLOWING INJECTION OF THOROTRAST

Time Interval (hrs.)	*Lymphocyte*		*Neutrophil*		*Blast Cells*	
	Mean ± S.E.	95% Range	Mean ± S.E.	95% Range	Mean ± S.E.	95% Range
(Control)	77.08 ± 2.94	71.12– 82.88	8.64 ± 1.83	4.98–12.30	2.50 ± 0.41	1.68– 3.32
1	53.00 ± 6.00	41.00– 65.00	39.50 ± 5.50	28.50–50.50	0.50 ± 0.50	0.00– 1.50
3	75.50 ± 12.50	50.50–100.50	17.50 ± 14.50	0.00–46.50	6.00 ± 2.00	2.00–10.00
5	53.66 ± 7.95	37.76– 69.56	32.66 ± 6.49	19.68–45.64	2.00 ± 0.57	0.86– 3.14
6	57.00 ± 2.80	51.40– 62.60	23.50 ± 2.50	18.50–28.50	10.50 ± 0.50	9.50–11.50
12*	60		10		1	
18–19	62.00 ± 5.00	52.00– 72.00	7.83 ± 4.95	0.00–17.73	4.00 ± 2.48	0.00– 9.96
24–26	89.00 ± 6.00	77.00–101.00	5.50 ± 3.50	0.00–12.50	3.00 ± 3.00	0.00– 9.00

*One blood sample available.

yama (1960) also referred to the mononuclear leukocytes in perch as transforming into macrophages, epithelioid cells, and Langhans giant cells. Jordan (1938) considered the intermediate lymphocytes of teleosts as lymphoid hemoblasts, serving as stem cells. Reports on teleost hematology describing monocytes, large or intermediate lymphocytes, and lymphoid hemoblasts, probably refer to transitional stages of similar cell types which may be considered, in most instances, to be lymphoid hemoblasts or lymphoblasts.

Since most immature cell types (lymphoid hemoblasts, lymphoblasts, and intermediate stages) observed in this study were all closely related morphologically, these cells have been grouped together as blast cells. The responsive cell types noted in the goldfish, following injection of the endocytic agent, were lymphocytes, immature and mature neutrophils, blast cells, and occasional eosinophils. The origin and function of the polymorphonuclear leukocytes in teleosts have been described. Lymphoid hemoblasts, originating from hemocytoblasts at hemopoietic sites, differentiate into both leukocytes and erythrocytes. Neutrophils and eosinophils have been described as active phagocytes. The origin and function of mononuclear leukocytes, however, are less definitive. Responses of blast cells and immature neutrophils have not been extensively reported previously.

Macrophage response was not noted in the fish studied. In the initial stages (first five hours) following injection, the neutrophil population consisted of immature stages comparable in maturation to mammalian promyelocytes, myelocytes, and metamyelocytes. Their appearance was concomitant with the drop in mature lymphocytes; however, no evidence of lymphocyte destruction was observed. The lymphocytes present were of the medium to large variety, and lymphoblasts were also prominent during this period. This blood picture reached a peak at six hours, after which the more usual picture of predominantly small lymphocytes and mature neutrophils was reestablished. Blast cell counts were variable because of the apparently rapid transformations occurring. Significant rises in blast cell populations were noted at three and six hours, coinciding with early lymphopenia and neutrophilia. The endocytic response was not typical of that seen in acute inflammatory reactions (also characterized by lymphopenia and neutrophilia) reported in a teleost (Weinreb, 1958), since in endocytosis the predominant cell types are both immature and mature polymorphonuclear and mononuclear leukocytes.

Changes in the peripheral blood picture may be attributed to changes in production and output of cells from hemopioetic sites and/or changes in cell populations in the circulation. It seems more likely, because of the small numbers of hemoblasts and the lack of evidence of cell division, that cellular transformations or differentiations are occurring in the circulation.

Previous reports related the mononuclear phagocytes to the hematological responses in fishes under various experimental conditions. Mesnil (1895) concluded, from a study of immunological response in goldfish which had been intraperitoneally injected with Anthrax bacilli, that resistance was due to phagocytosis by mononuclear cells. Metchnikoff (1905) found that guinea pig erythrocytes were quickly phagocytosed by mononuclears following injection into goldfish. Intraperitoneally injected trypan blue was described in carp mononuclear phagocytes by Wislocki (1917). Mackmull and Michels (1932), in studies of colloidal carbon absorption from the peritoneal cavity of the cutter, *Tautogolabrus adspersus,* found carbon in the polymorphonuclear leukocytes and wandering phagocytes. Monocytes, difficult to distinguish from histiocytes, were reported as active phagocytes. Some phagocytic activity was also attributed to blood and tissue eosinophils. Katz (1950), in a study of the silver salmon fingerling's response to bacterial infection, reported that neutrophils, lymphocytes, and monocytes were present, but only the mononuclear macrophages contained ingested bacteria. He considered the macrophage to be a specialized monocyte. Immature red cells, resembling basophilic normoblasts, were also present. Jakowska and Nigrelli (1953), in a study of bacteria infected guppies, noted phagocytic eosinophils and macrophages at the sites of skin lesions. Watson (1961) noted macrophages containing bacteria, neutrophils, and eosinophils at infection sites in goldfish. Both granulocytes and agranulocytes have been reported to be involved in fish blood cell responses, the macrophage which was prominent in bacterial infection studies, was less important under conditions of aseptic stimulus.

Mammalian studies indicate a more active role for the macrophage in both the normal and experimental animal. Rebuck *et al.* (1958) described transformation of lymphocytes into macrophages at acute inflammatory sites in man and noted that cells with features of the monocyte were observed prior to the appearance of the macrophage. Berman and Stulberg (1962), using primary cultures of human macrophages, studied transformations of original cells in cultures where mitoses were practically absent. Their system provided an experimental model for studying interrelationships between macro-

phages and lymphocytes. They observed, in leukocyte cultures, that granulocytes degenerate and disappear, while lymphocytes and monocytes became hypertrophied and underwent a series of changes resulting in macrophage morphology. Berman and Stulberg concluded that development of macrophages in cultures occurred, in the absence of mitoses, by transformation of lymphocytes, and probably small numbers of monocytes, of the original inocula. In a series of correlated morphological and biochemical studies on mammalian mononuclear phagocytes, Cohen *et al.* (1965a, 1965b, 1965c, 1966a, 1966b) have demonstrated, in both *in vitro* and *in vivo* systems, that monocyte-like cells differentiate into macrophages, and that endocytosis may be a major factor in macrophage differentiation and function.

The decrease in the number of small lymphocytes, and the increase in medium and large lymphocytes, blast cells, and immature neutrophils, suggest that there were cell-type transformations in the peripheral blood of the goldfish under study. As noted, the number of hemoblasts, normally present, is too small to account for the large numbers of immature cells observed. The presence of blast cells, mainly lymphoblasts, appears related to the altered lymphocyte picture. The multipotentiality of the lymphocyte has been extensively reported in both comparative and mammalian hematology. The function of the lymphocyte in the goldfish has not been precisely defined, yet this cell type constitutes over three-fourths of the circulating leukocytes. Its potential as a stem cell is considered highly probable.

It appears that changes in mononuclear cells are transient and reversible. The fate of neutrophils, on the other hand, seems irreversible. The large hemoblast, prominent in the early reactions, is active in the phagocytic defense mechanism as well as serving as a precursor of cell types partaking in the reaction. The blast cells, mainly lymphoid hemoblasts, which are prevalent during the first six hours following injection, are analogous to mammalian macrophages reported in other studies. It is possible that this cell is the result of "transformation" from the lymphocyte in the manner reported for mammalian macrophage formation.

The high incidence of blast cells in the circulation is attributed to the release from hemopoietic sites and transition from other mononuclear cell types. The neutrophil, which is the most active phagocyte, may originate from the blast cell, via lymphocytic transformations, or the hemoblast. A similar sequence was suggested for human bone marrow cells based on morphological and cytochemical studies of neutrophils (Ackerman, 1964). Hemopoietic activity of peripheral blood cells, such as lymphocytes, appears to be stimulated by various stress conditions, including aseptic endocytosis. In the teleost, the less mature blood cells have the potential for phagocytic activity as well as cell proliferation and differentiation.

Summary

1. The peripheral blood picture of the goldfish was studied to determine cell functions and analogies to mammalian cell types. Blood samples were collected at intervals, over a 26 hour period, from fish injected with Thorotrast. Stained blood smears were examined for qualitative and quantitative changes. The mean ± S. E. and 95% range were determined for all leukocytes.
2. Responsive elements were lymphocytes, immature and mature neutrophils, and a variety of blast cells. Lymphopenia and neutrophilia persisted for six hours. All neutrophilic maturation stages were present. The reaction decreased after 12 hours, and the blood picture began to normalize after 18 hours. The rare macrophages did not respond. Mononuclear phagocytes were limited to lymphocytes and blast cells.
3. The lymphoid hemoblasts are analogous to mammalian macrophages studied under similar conditions. Immature blood cells of the teleost have the potential for endocytosis as well as cell proliferation and differentiation. The probable roles of the lymphocyte in hemopoiesis and endocytosis are discussed.

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EXPLANATION OF THE PLATES

The following figures of Wright's-stained peripheral blood smears are magnified 1,560X.

Plate I

Fig. 1. One hour blood sample showing prevalence of immature granulocytes in early stages of reaction. Three immature neutrophils demonstrate stages in maturation comparable to mammalian early myelocyte (N^1), later myelocyte (N^2), and metamyelocyte (N^3). A medium-size lymphocyte (L) is present.

Fig. 2. One hour sample containing lymphoblast (B), lymphocyte (L), and late myelocytic stage (N) in neutrophil development.

Fig. 3. A large lymphoid hemoblast (B) showing extensive vacuolization (arrow), characteristic of blast cells noted in the three hour blood samples.

Fig. 4. A large lymphoid hemoblast (B) and an immature neutrophil (N) noted in a three hour sample. The blue-gray cytoplasm of the hemoblast closely resembles that of the mammalian monocyte. The neutrophil, comparable to a metamyelocytic neutrophil in morphology, exhibits two types of granulation: larger, deeply acidophilic granules towards the cell periphery; and smaller, neutral granules in the pale "hof" area.

Fig. 5. A lymphoblast (B) and examples of erythrocyte maturation in a three hour blood sample. The erythrocytic stages are represented by basophilic erythroblasts (E^1), a polychromatic erythroblast (E^2), and an erythrocyte (E^3).

Fig. 6. A dividing lymphocyte from a three hour blood sample.

Plate II

Fig. 7. A binucleate lymphocyte (L) and a mature neutrophil (N) in a three hour sample.

Fig. 8. A five hour sample demonstrating a small lymphoid hemoblast (B), with clear blue cytoplasm and extensive pseudopodia, a myelocytic neutrophil (N^1), and a later metamyelocytic neutrophil (N^2).

Fig. 9. A five hour sample with an immature neutrophil, comparable to a late myelocytic stage, showing abundant fine granulation filling the cytoplasm.

Fig. 10. A five hour sample demonstrating two metamyelocytic neutrophils. In addition to the fine granulation filling the cytoplasm, there are vacuoles which may contain large, granular inclusions (arrows).

Fig. 11. A late stage in lymphoblast development, in a six hour sample. This cell is smaller than less mature blast cells, with more condensed nuclear chromatin and deeper cytoplasmic basophilia.

Fig. 12. Lymphocytes in a six hour sample are of small and medium variety. The medium lymphocyte shows cytoplasmic blebbing; the large vacuole (arrow) noted in one of the blebs contains amorphous, acidophilic material.

Plate III

Fig. 13. Lymphocyte vacuoles in a six hour sample contain amorphous, acidophilic material and small aggregates of particulate matter (arrow). The cytoplasmic contour is irregular due to many small blebs.

Fig. 14. Early probasophilic stage of basophil maturation in an 18 hour sample. Large, very faintly stained granules fill the cytoplasm.

Fig. 15. An intermediate stage in basophil development, from the preceding 18 hour sample, showing specific granulation. At this stage, unlike the mature cell, the granules do not fill the cytoplasm nor obliterate the nuclear outline.

Fig. 16. A mature basophil, from the same 18 hour blood sample, demonstrates the typical morphology of this stage.

Fig. 17. A transitional stage (B) between the late lymphoblast and early lymphocyte is typical of mononuclear cells noted in the 24 hour blood samples. A small mature lymphocyte (L) is also present.

Fig. 18. Three mature lymphocytes, demonstrating typical morphology, are representative of the cell populations noted in blood samples after 24 hours.